

WHAT IS CLAIMED:

1. A thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.
2. A thermostable ligase according to claim 1, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.
3. A thermostable ligase according to claim 2, wherein, in the presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.
4. A thermostable ligase according to claim 3, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.
5. A thermostable ligase according to claim 1, wherein, in the presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a

- 38 -

mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

6. A thermostable ligase according to claim 5, wherein the
5 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

7. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif
10 where X is any amino acid.

8. A thermostable ligase according to claim 1, wherein the
thermostable ligase has a molecular weight of 78 to 81 kDa determined by SDS-
PAGE.
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9. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

10. A thermostable ligase having 50 fold higher fidelity than T4
20 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

11. A thermostable ligase according to claim 10, wherein, in the
25 presence of a Mn^{2+} cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation
30 junction at the base immediately adjacent to the ligation junction.

- 39 -

12. A thermostable ligase according to claim 11, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.
- 5 13. A thermostable ligase according to claim 10, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.
14. A thermostable ligase having an arginine adjacent its active
10 KXDG motif where X is any amino acid.
15. A thermostable ligase having, in the presence of a Mn^{2+} cofactor, a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a
15 target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.
16. An isolated DNA molecule encoding a thermostable ligase,
20 wherein the thermostable ligase has a 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.
- 25 17. An isolated DNA molecule according to claim 16, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a
30 mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.